

# SEQUENCE LISTING

<110> Enenkel, Barbara  
Fieder, Juergen  
Otto, Ralf  
Sautter, Kerstin  
Bergemann, Klaus

<120> Neomycin-Phosphotransferase Genes and Methods  
for the Selection of Recombinant Cells Producing  
High Levels of a Desired Gene Product

<130> 1/1411

<140> To be assigned

<141> 2003-11-26

<150> US 60/431,535

<151> 2002-12-06

<150> US 60/487,902

<151> 2003-07-17

<150> DE 102 56 081

<151> 2002-11-29

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<151> 2003-07-08

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<170> PatentIn Ver. 3.1

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&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Neomycin mutant E182G

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&lt;212&gt; PRT

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&lt;223&gt; Neomycin mutant E182G

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      20               25               30

Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe
      35               40               45

Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala
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Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val
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 Ala Thr Cys Pro Phe Asp His Gln Ala Lys His Arg Ile Glu Arg Ala  
                             130                            135                            140  
 Arg Thr Arg Met Glu Ala Gly Leu Val Asp Gln Asp Asp Leu Asp Glu  
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 Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala  
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 Leu Pro Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp  
                             195                            200                            205  
 Cys Gly Arg Leu Gly Val Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala  
                             210                            215                            220  
 Thr Arg Asp Ile Ala Glu Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe  
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 Leu Val Leu Tyr Gly Ile Ala Ala Pro Asp Ser Gln Arg Ile Ala Phe  
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      20             25             30

Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe
      35             40             45

Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala
      50             55             60

Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val
      65             70             75             80

Leu Asp Val Val Thr Glu Ala Gly Arg Asp Ala Leu Leu Leu Gly Glu
      85             90             95

Val Pro Gly Gln Asp Leu Leu Ser Ser His Leu Ala Pro Ala Glu Lys
      100            105            110

Val Ser Ile Met Ala Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro
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Ala Thr Cys Pro Phe Asp His Gln Ala Lys His Arg Ile Glu Arg Ala
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Arg Thr Arg Met Glu Ala Gly Leu Val Asp Gln Asp Asp Leu Asp Glu
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Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala
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Ser Met Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala Cys
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 210 215 220  
 Thr Arg Asp Ile Ala Glu Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe  
 225 230 235 240  
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Ala	Arg	Leu	Ser	Trp	Leu	Ala	Thr	Thr	Gly	Val	Pro	Cys	Ala	Ala	Val
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Leu	Asp	Val	Val	Thr	Glu	Ala	Gly	Arg	Asp	Trp	Leu	Leu	Leu	Gly	Glu
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Val	Ser	Ile	Met	Ala	Asp	Ala	Met	Arg	Arg	Leu	His	Thr	Leu	Asp	Pro
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Arg	Thr	Arg	Met	Glu	Ala	Gly	Leu	Val	Asp	Gln	Asp	Asp	Leu	Asp	Glu
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Ser	Met	Pro	Asp	Gly	Glu	Asp	Leu	Val	Val	Thr	His	Gly	Asp	Ala	Cys
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Leu	Pro	Asn	Ile	Met	Gly	Glu	Asn	Gly	Arg	Phe	Ser	Gly	Phe	Ile	Asp
		195					200					205			
Cys	Gly	Arg	Leu	Gly	Val	Ala	Asp	Arg	Tyr	Gln	Asp	Ile	Ala	Leu	Ala
	210					215					220				
Thr	Arg	Asp	Ile	Ala	Glu	Glu	Leu	Gly	Gly	Glu	Trp	Ala	Asp	Arg	Phe
	225					230					235				240
Leu	Val	Leu	Tyr	Gly	Ile	Ala	Ala	Pro	Asp	Ser	Gln	Arg	Ile	Ala	Phe
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<223> Neomycin mutant D227A

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Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe
          35                      40          45

Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala
 50                      55          60

Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val
 65                      70          75          80

Leu Asp Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly Glu
                85                      90          95

Val Pro Gly Gln Asp Leu Leu Ser Ser His Leu Ala Pro Ala Glu Lys
                100                     105          110

Val Ser Ile Met Ala Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro
 115                     120          125

Ala Thr Cys Pro Phe Asp His Gln Ala Lys His Arg Ile Glu Arg Ala
 130                     135          140
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 145 150 155 160  
 Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala  
 165 170 175  
 Ser Met Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala Cys  
 180 185 190  
 Leu Pro Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp  
 195 200 205  
 Cys Gly Arg Leu Gly Val Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala  
 210 215 220  
 Thr Arg Ala Ile Ala Glu Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe  
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			20					25					30			
Asp	Ala	Ala	Val	Phe	Arg	Leu	Ser	Ala	Gln	Gly	Arg	Pro	Val	Leu	Phe	
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Val	Lys	Thr	Asp	Leu	Ser	Gly	Ala	Leu	Asn	Glu	Leu	Gln	Asp	Glu	Ala	
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Cys	Gly	Arg	Leu	Gly	Val	Ala	Asp	Arg	Tyr	Gln	Asp	Ile	Ala	Leu	Ala	
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Leu	Val	Leu	Tyr	Gly	Ile	Ala	Ala	Pro	Asp	Ser	Gln	Arg	Ile	Ala	Phe	
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gcgcaggggc gcccggttct ttttgtcaag accgacctgt ccggtgccct gaatgaactg 180  
caagacgagg cagcgcggct atcgtggctg gccacgacgg gcgttccttg cgcagctgtg 240  
ctcgacgttg tactgaagc gggaaggac tggtgctat tgggcgaagt gccggggcag 300  
gatctcctgt catctcacct tgctcctgcc gagaaagtat ccatcatggc tgatgcaatg 360  
cggcggctgc atacgcttga tccggctacc tgcccattcg accaccaagc gaaacatcgc 420  
atcgagcgag cacgtactcg gatggaagcc ggtcttgtcg atcaggatga tctggacgaa 480  
gagcatcagg ggctcgcgcc agccgaactg ttcgccaggc tcaaggcgag catgcccac 540  
ggcgaggatc tcgtcgtgac ccatggcgat gcctgcttgc cgaatatcat ggtggaaaat 600  
ggccgctttt ctggattcat cgactgtggc cggctgggtg tggcggaccg ctatcaggac 660  
atagcgttgg ctaccctgta tattgctgaa gagcttggcg gcgaatgggc tgaccgcttc 720  
ctcgtgcttt acggtatcgc cgctcccgat tcgcagcgca tcgccttcta tcgccttctt 780  
ggcgagttct tctga 795

<210> 14  
<211> 264  
<212> PRT  
<213> Artificial sequence

<220>  
<223> Neomycin mutant D261G

<400> 14  
Met Ile Glu Gln Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val  
1 5 10 15  
Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser  
20 25 30  
Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe  
35 40 45  
Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala  
50 55 60  
Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val  
65 70 75 80  
Leu Asp Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly Glu

85								90				95			
Val	Pro	Gly	Gln	Asp	Leu	Leu	Ser	Ser	His	Leu	Ala	Pro	Ala	Glu	Lys
			100					105				110			
Val	Ser	Ile	Met	Ala	Asp	Ala	Met	Arg	Arg	Leu	His	Thr	Leu	Asp	Pro
		115					120					125			
Ala	Thr	Cys	Pro	Phe	Asp	His	Gln	Ala	Lys	His	Arg	Ile	Glu	Arg	Ala
	130					135					140				
Arg	Thr	Arg	Met	Glu	Ala	Gly	Leu	Val	Asp	Gln	Asp	Asp	Leu	Asp	Glu
145					150					155					160
Glu	His	Gln	Gly	Leu	Ala	Pro	Ala	Glu	Leu	Phe	Ala	Arg	Leu	Lys	Ala
				165					170					175	
Ser	Met	Pro	Asp	Gly	Glu	Asp	Leu	Val	Val	Thr	His	Gly	Asp	Ala	Cys
			180					185					190		
Leu	Pro	Asn	Ile	Met	Val	Glu	Asn	Gly	Arg	Phe	Ser	Gly	Phe	Ile	Asp
		195					200					205			
Cys	Gly	Arg	Leu	Gly	Val	Ala	Asp	Arg	Tyr	Gln	Asp	Ile	Ala	Leu	Ala
	210					215					220				
Thr	Arg	Asp	Ile	Ala	Glu	Glu	Leu	Gly	Gly	Glu	Trp	Ala	Asp	Arg	Phe
225					230					235					240
Leu	Val	Leu	Tyr	Gly	Ile	Ala	Ala	Pro	Asp	Ser	Gln	Arg	Ile	Ala	Phe
				245					250					255	
Tyr	Arg	Leu	Leu	Gly	Glu	Phe	Phe								
			260												

<210> 15  
 <211> 795  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Neomycin mutant D261N

<400> 15  
 atgattgaac aagatggatt gcacgcaggt tctccggccg cttgggtgga gaggctattc 60  
 ggctatgact gggcacaaca gacaatcggc tgctctgatg ccgccgtgtt ccggctgtca 120  
 gcgcaggggc gcccggttct ttttgtcaag accgacctgt ccggtgccct gaatgaactg 180  
 caagacgagg cagcgcggct atcgtggctg gccacgacgg gcgttccttg cgcagctgtg 240  
 ctcgacgttg tactgaagc gggaaggac tggctgctat tgggcgaagt gccggggcag 300  
 gatctcctgt catctcacct tgctcctgcc gagaaagtat ccatcatggc tgatgcaatg 360  
 cggcggctgc atacgcttga tccggctacc tgcccattcg accaccaagc gaaacatcgc 420  
 atcgagcgag cacgtactcg gatggaagcc ggtcttgtcg atcaggatga tctggacgaa 480  
 gagcatcagg ggctcgcgcc agccgaactg ttcgccaggc tcaaggcgag catgcccgcac 540

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ggcgaggatc tcgtcgtgac ccatggcgat gcctgcttgc cgaatatcat ggtggaaaat 600
ggccgctttt ctggattcat cgactgtggc cggctgggtg tggcggaccg ctatcaggac 660
atagcgtttg ctaccctgga tattgctgaa gagcttggcg gcgaatgggc tgaccgcttc 720
ctcgtgcttt acggtatcgc cgctcccgat tcgcagcgca tcgccttcta tcgccttctt 780
aacgatttct tctga 795

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```

<210> 16
<211> 264
<212> PRT
<213> Artificial sequence

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<220>
<223> Neomycin mutant D261N

```

```

<400> 16
Met Ile Glu Gln Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val
  1             5             10             15

Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser
      20             25             30

Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe
      35             40             45

Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala
      50             55             60

Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val
      65             70             75             80

Leu Asp Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly Glu
      85             90             95

Val Pro Gly Gln Asp Leu Leu Ser Ser His Leu Ala Pro Ala Glu Lys
      100            105            110

Val Ser Ile Met Ala Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro
      115            120            125

Ala Thr Cys Pro Phe Asp His Gln Ala Lys His Arg Ile Glu Arg Ala
      130            135            140

Arg Thr Arg Met Glu Ala Gly Leu Val Asp Gln Asp Asp Leu Asp Glu
      145            150            155            160

Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala
      165            170            175

Ser Met Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala Cys
      180            185            190

Leu Pro Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp
      195            200            205

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Cys Gly Arg Leu Gly Val Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala  
 210 215 220

Thr Arg Asp Ile Ala Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe  
 225 230 235 240

Leu Val Leu Tyr Gly Ile Ala Ala Pro Asp Ser Gln Arg Ile Ala Phe  
 245 250 255

Tyr Arg Leu Leu Asn Glu Phe Phe  
 260

<210> 17  
 <211> 795  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Neomycin mutant F240I

<400> 17  
 atgattgaac aagatggatt gcacgcaggt tctccggccg cttgggtgga gaggctattc 60  
 ggctatgact gggcacaaca gacaatcggc tgctctgatg ccgccgtgtt ccggctgtca 120  
 gcgcaggggc gcccggttct ttttgtcaag accgacctgt ccggtgccct gaatgaactg 180  
 caagacgagg cagcgcggtc atcgtggctg gccacgacgg gcgttccttg cgcagctgtg 240  
 ctcgacgttg tcaactgaagc gggaaggac tggctgctat tgggcgaagt gccggggcag 300  
 gatctcctgt catctcacct tgctcctgcc gagaaagtat ccatcatggc tgatgcaatg 360  
 cggcggtcgc atacgcttga tccggctacc tgcccattcg accaccaagc gaaacatcgc 420  
 atcgagcgag cacgtactcg gatggaagcc ggtcttgtcg atcaggatga tctggacgaa 480  
 gagcatcagg ggctcgcgcc agccgaactg ttcgccaggc tcaaggcgag catgcccgac 540  
 ggcgaggatc tcgtcgtgac ccatggcgat gcctgcttgc cgaatatcat ggtggaaaat 600  
 ggccgctttt ctggattcat cgactgtggc cggctgggtg tggcggaccg ctatcaggac 660  
 atagcgttgg ctaccggtga tattgctgaa gagcttggcg gcgaatgggc tgaccgcac 720  
 ctcgctgcttt acggtatcgc cgctcccgat tcgcagcgca tcgccttcta tcgccttctt 780  
 gacgagttct tctga 795

<210> 18  
 <211> 264  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> Neomycin mutant F240I

<400> 18  
 Met Ile Glu Gln Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val  
 1 5 10 15  
 Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser  
 20 25 30

Asp	Ala	Ala	Val	Phe	Arg	Leu	Ser	Ala	Gln	Gly	Arg	Pro	Val	Leu	Phe
	35						40					45			
Val	Lys	Thr	Asp	Leu	Ser	Gly	Ala	Leu	Asn	Glu	Leu	Gln	Asp	Glu	Ala
	50					55					60				
Ala	Arg	Leu	Ser	Trp	Leu	Ala	Thr	Thr	Gly	Val	Pro	Cys	Ala	Ala	Val
	65				70					75					80
Leu	Asp	Val	Val	Thr	Glu	Ala	Gly	Arg	Asp	Trp	Leu	Leu	Leu	Gly	Glu
				85					90					95	
Val	Pro	Gly	Gln	Asp	Leu	Leu	Ser	Ser	His	Leu	Ala	Pro	Ala	Glu	Lys
			100					105					110		
Val	Ser	Ile	Met	Ala	Asp	Ala	Met	Arg	Arg	Leu	His	Thr	Leu	Asp	Pro
		115					120					125			
Ala	Thr	Cys	Pro	Phe	Asp	His	Gln	Ala	Lys	His	Arg	Ile	Glu	Arg	Ala
	130					135					140				
Arg	Thr	Arg	Met	Glu	Ala	Gly	Leu	Val	Asp	Gln	Asp	Asp	Leu	Asp	Glu
	145				150					155					160
Glu	His	Gln	Gly	Leu	Ala	Pro	Ala	Glu	Leu	Phe	Ala	Arg	Leu	Lys	Ala
				165					170					175	
Ser	Met	Pro	Asp	Gly	Glu	Asp	Leu	Val	Val	Thr	His	Gly	Asp	Ala	Cys
			180					185					190		
Leu	Pro	Asn	Ile	Met	Val	Glu	Asn	Gly	Arg	Phe	Ser	Gly	Phe	Ile	Asp
		195					200					205			
Cys	Gly	Arg	Leu	Gly	Val	Ala	Asp	Arg	Tyr	Gln	Asp	Ile	Ala	Leu	Ala
	210					215					220				
Thr	Arg	Asp	Ile	Ala	Glu	Glu	Leu	Gly	Gly	Glu	Trp	Ala	Asp	Arg	Ile
	225				230					235					240
Leu	Val	Leu	Tyr	Gly	Ile	Ala	Ala	Pro	Asp	Ser	Gln	Arg	Ile	Ala	Phe
				245					250					255	
Tyr	Arg	Leu	Leu	Asp	Glu	Phe	Phe								
				260											

<210> 19

<211> 795

<212> DNA

<213> Artificial sequence

<220>

<223> Neomycin mutant E182D

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<400> 19
atgattgaac aagatggatt gcacgcaggt tctccggccg cttgggtgga gaggctattc 60
ggctatgact gggcacaaca gacaatcggc tgctctgatg ccgccgtggt ccggctgtca 120
gcgcaggggc gcccggttct ttttgtcaag accgacctgt ccggtgccct gaatgaactg 180
caagacgagg cagcgcggct atcgtggctg gccacgacgg gcgttccttg cgcagctgtg 240
ctcgacgttg tcaactgaagc gggaaggac tggtgctat tgggcgaagt gccggggcag 300
gatctcctgt catctcacct tgctcctgcc gagaaagtat ccatcatggc tgatgcaatg 360
cggcggctgc atacgcttga tccggctacc tgcccattcg accaccaagc gaaacatcgc 420
atcgagcgag cacgtactcg gatggaagcc ggtcttgctg atcaggatga tctggacgaa 480
gagcatcagg ggctcgcgcc agccgaactg ttcgccaggc tcaaggcgag catgcccgcac 540
ggcgatgac tcgtcgtgac ccatggcgat gcctgcttgc cgaatatcat ggtggaaaat 600
ggccgctttt ctggattcat cgactgtggc cggctgggtg tggcggaccg ctatcaggac 660
atagcgttgg ctaccctgta tattgctgaa gagcttggcg gcgaatgggc tgaccgcttc 720
ctcgtgcttt acggtatcgc cgctcccgat tcgcagcgca tcgccttcta tcgccttctt 780
gacgagttct tctga 795

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<210> 20
<211> 264
<212> PRT
<213> Artificial sequence

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<220>
<223> Neomycin mutant E182D

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<400> 20
Met Ile Glu Gln Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val
  1             5             10             15

Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser
      20             25             30

Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe
      35             40             45

Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala
      50             55             60

Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val
      65             70             75             80

Leu Asp Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly Glu
      85             90             95

Val Pro Gly Gln Asp Leu Leu Ser Ser His Leu Ala Pro Ala Glu Lys
      100             105             110

Val Ser Ile Met Ala Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro
      115             120             125

Ala Thr Cys Pro Phe Asp His Gln Ala Lys His Arg Ile Glu Arg Ala
      130             135             140

Arg Thr Arg Met Glu Ala Gly Leu Val Asp Gln Asp Asp Leu Asp Glu

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145		150		155		160
Glu His Gln Gly	Leu Ala Pro Ala	Glu Leu Phe Ala	Arg Leu Lys Ala			
	165		170			175
Ser Met Pro Asp	Gly Asp Asp Leu	Val Val Thr His	Gly Asp Ala Cys			
	180		185			190
Leu Pro Asn Ile	Met Val Glu Asn	Gly Arg Phe Ser	Gly Phe Ile Asp			
	195		200			205
Cys Gly Arg Leu	Gly Val Ala Asp	Arg Tyr Gln Asp	Ile Ala Leu Ala			
	210		215			220
Thr Arg Asp Ile	Ala Glu Glu Leu	Gly Gly Glu Trp	Ala Asp Arg Phe			
	225		230			235
Leu Val Leu Tyr	Gly Ile Ala Ala	Pro Asp Ser Gln	Arg Ile Ala Phe			
	245		250			255
Tyr Arg Leu Leu	Asp Glu Phe Phe					
	260					

<210> 21  
 <211> 795  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Neomycin mutant D227G

<400> 21  
 atgattgaac aagatggatt gcacgcaggt tctccggccg cttgggtgga gaggctattc 60  
 ggctatgact gggcacaaca gacaatcggc tgctctgatg ccgccgtgtt ccggctgtca 120  
 gcgcaggggc gcccggttct ttttgtcaag accgacctgt ccggtgccct gaatgaactg 180  
 caagacgagg cagcgcggtc atcgtggctg gccacgacgg gcgttccttg cgcagctgtg 240  
 ctcgacgttg tcaactgaagc gggaagggac tggctgctat tgggcgaagt gccggggcag 300  
 gatctcctgt catctcacct tgctcctgcc gagaaagtat ccatcatggc tgatgcaatg 360  
 cggcggctgc atacgcttga tccggctacc tgcccattcg accaccaagc gaaacatcgc 420  
 atcgagcgag cacgtactcg gatggaagcc ggtcttgtcg atcaggatga tctggacgaa 480  
 gagcatcagg ggctcgcgcc agccgaactg ttcgccaggc tcaaggcgag catgcccgac 540  
 ggcgaggatc tcgtcgtgac ccatggcgat gcctgcttgc cgaatatcat ggtggaaaat 600  
 ggccgctttt ctggattcat cgactgtggc cggctgggtg tggcggaccg ctatcaggac 660  
 atagcgttgg ctaccctgtg tattgctgaa gagcttggcg gcgaatgggc tgaccgcttc 720  
 ctctgtcttt acggtatcgc cgctcccgat tcgcagcgca tcgccttcta tcgccttctt 780  
 gacgagttct tctga 795

<210> 22  
 <211> 264  
 <212> PRT  
 <213> Artificial sequence

<220>

<223> Neomycin mutant D227G

<400> 22

Met	Ile	Glu	Gln	Asp	Gly	Leu	His	Ala	Gly	Ser	Pro	Ala	Ala	Trp	Val	
1				5					10					15		
Glu	Arg	Leu	Phe	Gly	Tyr	Asp	Trp	Ala	Gln	Gln	Thr	Ile	Gly	Cys	Ser	
			20					25					30			
Asp	Ala	Ala	Val	Phe	Arg	Leu	Ser	Ala	Gln	Gly	Arg	Pro	Val	Leu	Phe	
		35					40					45				
Val	Lys	Thr	Asp	Leu	Ser	Gly	Ala	Leu	Asn	Glu	Leu	Gln	Asp	Glu	Ala	
	50					55					60					
Ala	Arg	Leu	Ser	Trp	Leu	Ala	Thr	Thr	Gly	Val	Pro	Cys	Ala	Ala	Val	
65					70					75					80	
Leu	Asp	Val	Val	Thr	Glu	Ala	Gly	Arg	Asp	Trp	Leu	Leu	Leu	Gly	Glu	
				85					90					95		
Val	Pro	Gly	Gln	Asp	Leu	Leu	Ser	Ser	His	Leu	Ala	Pro	Ala	Glu	Lys	
			100					105					110			
Val	Ser	Ile	Met	Ala	Asp	Ala	Met	Arg	Arg	Leu	His	Thr	Leu	Asp	Pro	
		115					120					125				
Ala	Thr	Cys	Pro	Phe	Asp	His	Gln	Ala	Lys	His	Arg	Ile	Glu	Arg	Ala	
	130					135					140					
Arg	Thr	Arg	Met	Glu	Ala	Gly	Leu	Val	Asp	Gln	Asp	Asp	Leu	Asp	Glu	
145					150					155					160	
Glu	His	Gln	Gly	Leu	Ala	Pro	Ala	Glu	Leu	Phe	Ala	Arg	Leu	Lys	Ala	
				165					170					175		
Ser	Met	Pro	Asp	Gly	Glu	Asp	Leu	Val	Val	Thr	His	Gly	Asp	Ala	Cys	
			180					185					190			
Leu	Pro	Asn	Ile	Met	Val	Glu	Asn	Gly	Arg	Phe	Ser	Gly	Phe	Ile	Asp	
		195					200					205				
Cys	Gly	Arg	Leu	Gly	Val	Ala	Asp	Arg	Tyr	Gln	Asp	Ile	Ala	Leu	Ala	
	210					215					220					
Thr	Arg	Gly	Ile	Ala	Glu	Glu	Leu	Gly	Gly	Glu	Trp	Ala	Asp	Arg	Phe	
225					230					235					240	
Leu	Val	Leu	Tyr	Gly	Ile	Ala	Ala	Pro	Asp	Ser	Gln	Arg	Ile	Ala	Phe	
				245					250					255		
Tyr	Arg	Leu	Leu	Asp	Glu	Phe	Phe									
				260												

<210> 23  
 <211> 795  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Neomycin mutant D190G

<400> 23  
 atgattgaac aagatggatt gcacgcaggt tctccggccg cttgggtgga gaggctattc 60  
 ggctatgact gggcacaaca gacaatcggc tgctctgatg ccgccgtggt ccggctgtca 120  
 gcgcaggggc gcccggttct ttttgtcaag accgacctgt ccggtgccct gaatgaactg 180  
 caagacgagg cagcgcggct atcgtggctg gccacgacgg gcgttccttg cgcagctgtg 240  
 ctgcagcttg tcaactgaagc gggaagggac tggctgctat tgggcgaagt gccggggcag 300  
 gatctcctgt catctcacct tgctcctgcc gagaaagtat ccatcatggc tgatgcaatg 360  
 cggcggctgc atacgcttga tccggctacc tgcccattcg accaccaagc gaaacatcgc 420  
 atcgagcgag caggtactcg gatggaagcc ggtcttgctg atcaggatga tctggacgaa 480  
 gagcatcagg ggctcgcgcc agccgaactg ttcgccaggc tcaaggcgag catgcccagc 540  
 ggcgaggatc tcgtcgtgac ccatggcggt gcctgcttgc cgaatatcat ggtggaaaat 600  
 ggccgctttt ctggattcat cgactgtggc cggctgggtg tggcggaccg ctatcaggac 660  
 atagcgttgg ctaccctgta tattgctgaa gagcttggcg gcgaatgggc tgaccgcttc 720  
 ctcgtgcttt acggtatcgc cgctcccgat tcgcagcgca tcgccttcta tcgccttctt 780  
 gacgagttct tctga 795

<210> 24  
 <211> 264  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> Neomycin mutant D190G

<400> 24  
 Met Ile Glu Gln Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val  
 1 5 10 15  
 Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser  
 20 25 30  
 Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe  
 35 40 45  
 Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala  
 50 55 60  
 Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val  
 65 70 75 80  
 Leu Asp Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly Glu  
 85 90 95

Val Pro Gly Gln Asp Leu Leu Ser Ser His Leu Ala Pro Ala Glu Lys  
 100 105 110  
 Val Ser Ile Met Ala Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro  
 115 120 125  
 Ala Thr Cys Pro Phe Asp His Gln Ala Lys His Arg Ile Glu Arg Ala  
 130 135 140  
 Arg Thr Arg Met Glu Ala Gly Leu Val Asp Gln Asp Asp Leu Asp Glu  
 145 150 155 160  
 Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala  
 165 170 175  
 Ser Met Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Gly Ala Cys  
 180 185 190  
 Leu Pro Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp  
 195 200 205  
 Cys Gly Arg Leu Gly Val Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala  
 210 215 220  
 Thr Arg Asp Ile Ala Glu Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe  
 225 230 235 240  
 Leu Val Leu Tyr Gly Ile Ala Ala Pro Asp Ser Gln Arg Ile Ala Phe  
 245 250 255  
 Tyr Arg Leu Leu Asp Glu Phe Phe  
 260

<210> 25  
 <211> 795  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Neomycin mutant D208G

<400> 25  
 atgattgaac aagatggatt gcacgcaggt tctccggccg cttgggtgga gaggctattc 60  
 ggctatgact gggcacaaca gacaatcggc tgctctgatg ccgccgtggt ccggctgtca 120  
 gcgcaggggc gcccggttct ttttgtcaag accgacctgt ccggtgccct gaatgaactg 180  
 caagacgagg cagcgcggtc atcgtggctg gccacgacgg gcgttccttg cgcagctgtg 240  
 ctcgacgttg tcaactgaagc gggaagggac tggctgctat tgggcgaagt gccggggcag 300  
 gatctcctgt catctcacct tgctcctgcc gagaaagtat ccatcatggc tgatgcaatg 360  
 cggcgggtgc atacgcttga tccggctacc tgcccattcg accaccaagc gaaacatcgc 420  
 atcgagcgag cacgtactcg gatggaagcc ggtcttgtcg atcaggatga tctggacgaa 480  
 gagcatcagg ggctcgcgcc agccgaactg ttccgccagg tcaaggcgag catgcccgac 540  
 ggcgaggatc tcgtcgtgac ccatggcgat gcctgcttgc cgaatatcat ggtggaaaat 600  
 ggccgctttt ctggattcat cggctgtggc cggctgggtg tggcggaccg ctatcaggac 660

atagcgttgg ctacccgtga tattgctgaa gagcttggcg gcgaatgggc tgaccgcttc 720  
 ctcgtgcttt acggtatcgc cgctcccgat tcgcagcgca tcgccttcta tcgccttctt 780  
 gacgagttct tctga 795

<210> 26  
 <211> 264  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> Neomycin mutant D208G

<400> 26  
 Met Ile Glu Gln Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val  
 1 5 10 15  
 Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser  
 20 25 30  
 Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe  
 35 40 45  
 Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala  
 50 55 60  
 Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val  
 65 70 75 80  
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 Val Ser Ile Met Ala Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro  
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 Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala  
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 Ser Met Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala Cys  
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